P1 lysates

- To a 150ml flask add:
  - 25µl 1M $CaCl_2$
  - 25µl overnight culture
  - 5ml LB
  - 2µl P1 stock (approximately 10^8 pfu/ml)
  - 50µl 1M $MgSO_4$
- Let grow shaking at 37˚C until you see complete lysis (usually 4 hours)

- Add 10 drops of chloroform and vortex
- Let stand 10 minutes at room temperature
- Transfer lysate to Eppendorf tubes
- Centrifuge 2min
- Transfer supernatant to a new tube
- Add 2 drops of chloroform and vortex
- Let stand 10 min at RT
- Centrifuge 2min
- Transfer supernatant to a new tube, leaving the chloroform behind
- Centrifuge 2min
- Transfer supernatant to new tube.

P1 transduction

Note: if using P1 lysates from the fridge add 3 drops of chloroform, vortex and let stand 10min at RT then centrifuge 2mins and transfer to a new tube.

Remember to do controls where you don't add any lysates!

- Centrifuge overnight culture
- Resuspend cells in 1/2 volume (e.g. 1ml overnight, resuspend in 500µl) MC buffer
- Add 10-100µl P1 lysate (20µl mostly works) to 100µl cells
- Incubate 20mins at 37˚C
- Add 150µl 1M NaCitrate
- Add 1ml LB
- Incubate 1 hour at 37˚C
- Centrifuge
- Resuspend pellet in 0,1ml LB (okay to leave some supernatant and resuspend in that) and spread all on a selective plate

Also spot 5µl P1 lysate, 5µl MC buffer, 5µl LB and 5µl NaCitrate onto a LB plate containing <u>no</u> antibiotics to check for contamination.

MC buffer:

- 0,1M $MgSO_4$	100µl 1M $MgSO_4$ for 1ml MC
- 0,005M $CaCl_2$	50µl 0,1M $CaCl_2$ for 1ml MC
- H_2O	850µl for 1ml MC